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(54) Title: CRYSTAL STRUCTURE OF BETA-SITE APP CLEAVING ENZYME (BACE) AND USE THEREOF

(57) Abstract: Disclosed and claimed are novel BACE proteins, crystal structures thereof, nucleic acid molecules therefor, and methods for making and using and uses of the same, especially for ascertaining inhibitors of BACE; and thus, disclosed and claimed too are inhibitors of BACE and methods of making and using the same.

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TITLE OF THE INVENTION

NOVEL BACE PROTEINS, NUCLEIC ACID MOLECULES THEREFOR, NOVEL CRYSTAL STRUCTURE OF NOVEL BACE PROTEINS, AND METHODS FOR MAKING AND USING

FIELD OF THE INVENTION

This invention relates generally to structural studies of the soluble Beta-site APP cleaving enzyme (BACE) catalytic domain (e.g., the aspartyl protease domains of BACE) and the corresponding structural information obtained by X-ray crystallography.

Moreover, the present invention relates to any one or more of:

A catalytic domain of BACE, or a form of BACE that is suitable for crystallization with the correct disulphide bonding that eliminates the need for refolding and/or apo-BACE crystals which are BACE crystals with no ligand bound, regardless of the source of the BACE) and/or apo-BACE crystals which are capable of being soaked with ligand to give complexes and/or a crystalline form of BACE having crystals that are grown at or near the physiological pH of the enzyme such as between about pH 5.6 and about pH 5.8 and/or having a space group of C2 and cell dimensions of a= 236.63Å or 236.63Å ± standard deviation (0.2Å) or 236.63Å ± cell variability of 3Å, b= 105.02Å or 105.02Å ± standard deviation (0.2Å) or 105.02Å ± cell variability of 3Å, and c= 62.59Å or 62.59Å ± standard deviation (0.2Å) or 62.59Å ± cell variability of 3Å and β=101.32° or 101.32° ± standard deviation (0.2°) between 101° and 108° with the asymmetric unit of the crystal containing three copies of BACE (e.g., from growth in the presence of OM99-2) or cell dimensions a= 238.3Å or 238.3Å ± standard deviation (0.2Å) or 238.3Å ± cell variability of 3Å, b= 107.4Å ± standard deviation (0.2Å) or 107.4Å ± cell variability of 3Å, and c= 60.4Å or 60.4Å ± standard deviation (0.2Å) or 60.4Å ± cell variability of 3Å and β=101.89° or 101.89° ± standard deviation (0.2°) or between 101° and 108° (e.g., from crystals grown in the absence of OM99-2) and/or having an X-ray diffraction pattern corresponding to or resulting from any or all of the foregoing;

Apo-BACE crystals that can be soaked, e.g., with ligands such as inhibitory or modulatory ligands, to give complexes, such as protein-ligand complexes;

A crystalline form of BACE or a BACE that has an active site containing one or more ligands other than the natural substrate or the substrate that occurs naturally or physiologically within the active site or apo-BACE crystals with no ligand bound, regardless of the source of the BACE; for instance, for use in rational drug design, as well as methods for ligand screening and design by X-ray crystallography;

WHAT IS CLAIMED IS:

1. A catalytic domain of BACE or a form of BACE that is suitable for crystallization with the correct disulphide bonding that eliminates the need for refolding and/or an apo-BACE crystal or an apo-BACE crystal that can be soaked to give complexes and/or a crystalline form of BACE having crystals that are grown at or near the physiological pH of the enzyme or between about pH 5.6 and about pH 5.8 and/or a BACE crystal having a space group of C2 and/or a BACE crystal having cell dimensions of a= 236.63Å or 236.63Å ± standard deviation (0.2Å) or 236.63Å ± 3.0Å, b= 105.02Å or 105.02Å ± standard deviation (0.2Å) or 105.02Å ± 3.0Å, and c= 62.59Å or 62.59Å ± standard deviation (0.2Å) or 62.59Å ± 3.0Å and β=101.32° or 101.32° ± standard deviation (0.2°) or between 101° and 108° with the asymmetric unit of the crystal containing three copies of BACE or cell dimensions a= 238.3Å or 238.3Å ± standard deviation (0.2Å) or 238.3Å ± 3.0Å, b= 107.4Å ± standard deviation (0.2Å) or 107.4Å ± 3.0Å, and c= 60.4Å or 60.4Å ± standard deviation (0.2Å) or 60.4Å ± 3.0Å and β=101.89° or 101.89° ± standard deviation (0.2°) or between 101° and 108° and/or having an X-ray diffraction pattern corresponding to or resulting from any or all of the foregoing and/or having an X-ray diffraction pattern corresponding to or resulting from any or all of the foregoing; and/or having a space group transition from C2 to $P2_1$ together with an increase in the number of copies of the molecule in the asymmetric unit, while the cell dimensions and the packing of the $P2_1$ form are closely related to those of the C2 crystal form, on soaking the apo-BACE crystal with a ligand; and/or a BACE crystal having a resolution better than 3 Å; and/or a BACE crystal having the structure defined by the co-ordinates of Table 5.

2. A BACE crystal having the structure defined by the co-ordinates of Table 5.

3. An apo-BACE crystal grown at or near the physiological pH of the enzyme.

4. An apo-BACE crystal or an apo-BACE crystal that can be soaked to give complexes.

5. A crystalline form of BACE or a functional portion thereof having crystals that are grown at or near the physiological pH of the enzyme.

6. The crystalline form of BACE or functional portion thereof of claim 6 wherein the crystals are grown at a pH between about pH 5.6 and about pH 5.8

7. A crystalline form of BACE or a functional portion thereof having a space group of C2 and cell dimensions of a= 236.63Å or 236.63Å ± standard deviation (0.2Å) 236.63Å ± 3.0Å, b= 105.02Å or 105.02Å ± standard deviation (0.2Å) or 105.02Å ± 3.0Å, and c= 62.59Å or 62.59Å ± standard deviation (0.2Å) or 62.59Å ± 3.0Å and β=101.32° or 101.32° ± standard deviation (0.2°) or between 101° and 108° with the asymmetric unit of the crystal containing three copies of BACE or cell dimensions a= 238.3Å or 238.3Å ± standard deviation (0.2Å) or 238.3Å ± 3.0Å, b= 107.4Å or 107.4Å ± standard deviation (0.2Å) or 107.4Å ± 3.0Å, and c= 60.4Å or 60.4Å ± standard deviation (0.2Å) or 60.4Å ± 3.0Å and β=101.89° or 101.89° ± standard deviation (0.2°) or between 101° and 108° and/or having an X-ray diffraction pattern corresponding to or resulting from any or all of the foregoing and/or having an X-ray diffraction pattern corresponding to or resulting from any or all of the foregoing and/or having a space group transition from C2 to $P2_1$ together with an increase in the number of copies of the molecule in the asymmetric unit, while the cell dimensions and the packing of the $P2_1$ form are closely related to those of the C2 crystal form, on soaking the apo-BACE crystal with a ligand.

8. A crystalline form of BACE or a functional portion thereof that has an active site containing one or more ligands other than the natural substrate or the substrate that occurs naturally or physiologically within the active site.

9. A method for ligand screening or identification comprising exposing the BACE crystals of any one of claims 2-8 to one or more test samples, and determining whether a ligand-BACE complex is formed.

10. The method of claim 9 wherein the BACE protein or functional portion thereof is exposed to the test samples by co-crystallizing the BACE protein or functional portion thereof in the presence of the one or more test samples.

11. The method of claim 9 wherein the BACE of claims 2-8 is soaked in a solution of one or more test samples

12. A computer-assisted method for identifying or designing potential ligands to fit within the catalytic domain of BACE or a functional portion thereof:

comprising using a programmed computer comprising a processor, a data storage system, an input device, and an output device, the steps of: (a) inputting into the programmed computer through said input device data comprising the three-dimensional co-ordinates of a subset of the